U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-284 756

Survival and Reproduction of 'Gammarus lacustris and G. pseudolimnaeus' Under Two Experimental Conditions

U.S. Environmental Research Lab -Duluth, Minn

25 Jan 78

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3.
EPA-600/J-78-027	JOURNAL ARTICLE	PB 284 756

4. TITLE AND SUBTITLE	5. REPORT DATE
Survival and Reproduction of Gammarus lacustris and G. pseudolimnaeus Under Two Experimental Conditions	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Barbara J. Halligan and John G. Eaton	
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, MN 55804	10. PROGRAM ELEMENT NO. 1BA608 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Same	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/03

15. SUPPLEMENTARY NOTES REFERENCE: Halligan, Barbara J., and John G. Eaton. 1978. Survival and Reproduction of Gammarus lacustris and G. pseudolimnaeus Under Two Experimental Conditions. Prog. Fish-Cult. 40(2): 59-62.

16. ABSTRACT

Life-cycle tests on the survival and reproduction of the amphipods Gammarus lacustris and G. pseudolimnaeus were conducted under two nontoxicant experimental conditions: with several amphipods per tank and with individual pairs per tank. Juvenile and adult survival of the two species was similar with several amphipods per tank, but was higher for G. lacustris among the reproducing individual pairs. In the multiple-animal tanks G. lacustris females had larger broods than did G. pseudolimnaeus females and both species produced more young per female and more broods per female than in the tanks with individual pairs. Our results indicate that these amphipods may be reared more successfully with several animals per tank than with only two. Of the two species G. lacustris may be preferred for use in chronic toxicity tests because of its greater fecundity, larger size, and wider distribution.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Crustacea Life span Population growth Invertebrates	Amphipods Test procedures	06-C

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	45
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-78-027
JOURNAL ARTICLE

Survival and Reproduction of *Gammarus lacustris* and *G. pseudolimnaeus* Under Two Experimental Conditions

Barbara J. Halligan and John G. Eaton

U. S. Environmental Protection Agency, Environmental Research Laboratory–Duluth
6201 Congdon Boulevard, Duluth, Minnesota 55804

ABSTRACT: Life-cycle tests on the survival and reproduction of the amphipods *Gammarus lacustris* and *G. pseudolimnaeus* were conducted under two nontoxicant experimental conditions: with several amphipods per tank and with individual pairs per tank. Juvenile and adult survival of the two species was similar with several amphipods per tank, but was higher for *G. lacustris* among the reproducing individual pairs. In the multiple-animal tanks *G. lacustris* females had larger broods than did *G. pseudolimnaeus* females and both species produced more young per female and more broods per female than in the tanks with individual pairs. Our results indicate that these amphipods may be reared more successfully with several animals per tank than with only two. Of the two species, *G. lacustris* may be preferred for use in chronic toxicity tests because of its greater fecundity, larger size, and wider distribution.

Amphipods have been used frequently in evaluations of aquatic hazards (Sanders 1969; Arthur and Leonard 1970; Arthur and Eaton 1971; Sanders 1972; Arthur et al. 1973; Macek et al. 1976a,b) because they are representative of an important class of fish-food organisms and because they are known to be sensitive to many types of water contaminants (National Academy of Sciences/Engineering 1973). *Gammarus pseudolimnaeus, G. lacustris*, and *G. fasciatus* have been the most commonly used amphipods in these toxicity tests.

Chronic toxicity tests at our laboratory with *G. pseudolimnaeus* and elsewhere with *G. lacustris* resulted in greater than expected variation in reproduction and survival, suggesting the need for additional studies. We therefore conducted long-term rearing tests with *G. lacustris* and *G. pseudolimnaeus*. We compared and evaluated survival and reproduction under two nontoxicant test conditions to learn more about the life histories of these species and to better understand the causes of variation for each species and how to deal with these variations in life-cycle tests with environmental contaminants.

Methods

Adult *Gammarus lacustris* were collected from the Moose River at Sturgeon Lake, Minnesota, and *G. pseudolimnaeus* from the Eau Claire River at Gordon, Wisconsin. Both species were placed in holding tanks in the laboratory for at least 10 days at 17 ± 1°C before experimental use. When the females became visibly gravid (abdomen swollen and pink), they were isolated in 400-ml beakers containing about 250 ml of water and held until the young were released. The young were counted and returned to the beakers, with a small (1-2 cm) birch leaf for food, until enough were collected to start the test (1-20 days). To prevent depletion of oxygen and to maintain water quality, we changed about half the water in the beakers daily.

We used modified intermittent flow water-delivery systems (Mount and Brungs 1967) to deliver Lake Superior water to the test tanks at a rate of about 8 tank volumes every 24 h, and the systems were so connected that water was supplied to all tanks simultaneously. The tests were conducted at 17 ± 1°C; Smith (1973) found this temperature to be optimal for these amphipod species.

All the amphipods were fed birch and aspen leaves that had previously been soaked in lake water for 30 days. This food was the best of several tested in growth and reproduction experiments (J. W. Arthur and B. J. Halligan, Environmental Research Laboratory–Duluth, unpublished data).

Gro-Lux[1] and Duro-test Vitalite bulbs were suspended so they provided 100 lumens at the water surface: a 16-h-light:8-h-dark photoperiod was used.

Water hardness, pH, alkalinity, and acidity (American Public Health Association et al. 1976) were monitored weekly, and dissolved oxygen was monitored twice each week. Dissolved oxygen ranged from 10.8 to 17.6 mg/l. The water in the test tanks was supersaturated for 3-4 days during the second month of the experiment; bubbles formed

[1]Mention of brand names or manufacturers is for identification only and does not imply endorsement by the U.S. Environmental Protection Agency.

on the amphipods and hampered their swimming ability. The supersaturation was corrected and no apparent harm to the animals resulted. Averages and ranges (mg/l) of other characteristics included total hardness, 44.5 (43.0 - 46.0) and total alkalinity, 41.7 (41.0 - 42.0) —both as $CaCO_3$ — and acidity, 1.4 (1.0 - 1.9). The pH range was 7.3 - 7.7.

Experimental Designs

We used two experimental designs, one employing groups of animals (test 1) and the other, pairs of animals (test 2).

Test 1: Twenty *Gammarus lacustris*, 1-20 days old, were randomly placed in each of eight 19-liter (5-gal) tanks containing 14.8 liters of lake water; the same procedure was followed with *G. pseudolimnaeus* of the same age (total, 16 tanks). Each tank was provided with four pre-soaked birch leaves and four pre-soaked aspen leaves as food; as these leaves were consumed more were added. Surviving animals were counted every 30 days, and each tank was impartially thinned to 15 amphipods at 60 days. Amphipods began to copulate after about 12 weeks, and as females became visibly gravid, they were isolated individually in 400-ml beakers containing about 250 ml of test water. After recording the numbers of released young, we discarded them and returned the adults to their respective tanks. The test was terminated after 20 weeks and the sex of the adults was determined. The body length of straightened adults was measured from the posterior end of the head capsule to the anterior end of the telson.

Test 2: Under the same conditions of water delivery, temperature, and light, tests were run with individual pairs of both species. Forty *G. lacustris*, 30-39 days old, and 40 *G. pseudolimnaeus*, 21-24 days old, were first placed in four 19-liter tanks (20 per tank) and held until maturity. The animals were then observed several times daily and as soon as a pair copulated, they were placed in a 9.5-liter (2.5-gal) tank containing 7.6 liters of water. The experiment was started when there was one pair of *G. lacustris* in each of 12 tanks and one pair of *G. pseudolimnaeus* in each of 12 tanks (total, 24 tanks). Each tank was provided with four pre-soaked leaves (two birch and two aspen) as food and more leaves were added as needed. Because of lack of copulation and high early mortality of *G. pseudolimnaeus* in the individual tanks, the entire stock of this species was removed from the tanks and discarded. A new stock of juveniles, estimated by their size to be 30-60 days old, was collected, acclimated to laboratory conditions for 10 days, and used to restart the test. All isolated pairs were observed daily and when the females became visibly gravid, they were treated as described for test 1 to determine fecundity. The test was continued until one of the adult animals in each tank had died.

For each test, survival and reproduction data for the two species were compared statistically for significant differences by a one-way analysis of variance and a two-tailed t-distribution (Steel and Torrie 1960).

Results

In test 1 (several amphipods per tank), survival of both species was low during the first 30 days but there was no significant difference ($P = 0.05$) between them at any time interval (Table 1). The slightly reduced survival of both species from 120 days to the end of the test may have been due to senescence.

Table 1. Percentage survival during various time intervals of Gammarus lacustris *and* G. pseudolimnaeus *reared in groups (test 1).*

Time interval (days)	*G. lacustris*		*G. pseudolimnaeus*	
	Mean	Standard deviation	Mean	Standard deviation
At 30	70.6	18.2	58.8	8.4
31-60	95.1	6.9	95.0	7.4
61-90	94.1	8.1	96.8	4.6
91-120	97.1	5.6	99.0	2.8
121-140[a]	91.8	10.2	90.9	9.5

[a] End of test.

In test 1, *G. lacustris* produced more young than did *G. pseudolimnaeus* ($P = 0.01$) in 140 days (Table 2). Although there was no significant difference ($P = 0.05$) in the number of broods released by the two species, *G. lacustris* produced a greater number ($P = 0.01$) of young per brood.

Since the mortality of both species was low during the reproductive period of test 1 (about 12 weeks) we assumed that there was little difference in the mortality of females of the two species. Therefore, we used the number of females at the end of the test in calculations of reproductive success. On this basis there were no significant differences ($P = 0.05$) in the number of young per female of the two species (Table 2).

In test 2 (one pair of animals per tank), *G. lacustris* released a significantly greater number of young per female and produced more broods per female ($P = 0.01$) than did *G. pseudolimnaeus*, calculated on the basis of the number of starting females (Table 2). No significant difference ($P = 0.05$) was observed in the number of young per brood. The difference in reproductive capacity of the two species can best be explained by the difference of 45 days in their mean reproduction periods (108 days for *G. lacustris* vs. 63 days for *G. pseudolimnaeus*).

For comparison with the results of test 1, survival and reproduction after 20 weeks were determined for test 2 (Table 2). Although survival was about the same for the two species in test 1 between 90 and 140 days (corresponding approximately to the period in test 2 from the introduction of the individual pairs into the test tanks until they were 20 weeks old), survival among individual pairs (test 2) appeared to be much better for *G. lacustris* (none dead) than for *G. pseudolimnaeus* (five dead). However, the sample size in test 2 was much smaller than in test 1. In test 2 after 20

Table 2. Mean and standard deviation (in parentheses) of reproductive characteristics of Gammarus lacustris *and* G. pseudolimnaeus *reared in groups (test 1) and in pairs (test 2).*

Rearing method and species	Reproductive characteristics				
	No. of young	No. of broods	Young per brood	Broods per female	Young per female
In groups					
G. lacustris	268 (85)	9.9 (3.0)	27.3 (9.2)	1.8 (0.4)[a]	48.8 (16.0)[a]
G. pseudolimnaeus	168 (65)	7.6 (3.2)	22.0 (8.4)	2.3 (0.7)[a]	46.2 (16.0)[a]
In pairs[b]					
G. lacustris	—	—	26.8 (9.0)	3.5 (1.4)	94 (43)
G. pseudolimnaeus	—	—	30.4 (11.9)	1.3 (1.6)	41 (47)
In pairs[c]					
G. lacustris	—	—	24.7 (6.8)	1.3 (0.6)	30 (17)
G. pseudolimnaeus	—	—	27.7 (9.7)	1.3 (0.5)	33 (10)

[a] On the basis of the total number of females in all test chambers at the end of the test (44 *G. lacustris* and 29 *G. pseudolimnaeus*).

[b] Reproduction up to the time of death of one partner.

[c] Reproduction after 20 weeks (based on 12 live pairs of *G. lacustris* and 7 of *G. pseudolimnaeus*).

weeks, the numbers of young per surviving pair, broods per surviving pair, and young per brood were about the same (Table 2) for both species (no significant difference at $P = 0.05$). The number of young per surviving pair vs. starting pairs and the number of broods per surviving pair was lower for both species in test 2 than in test 1 (Table 2), but the number of young per brood was similar for the two test species in both tests.

Discussion

In test 1, survival of both *G. lacustris* and *G. pseudolimnaeus* was poorer during the first 30 days than it was from 31 to 140 days (when the test ended). We observed flatworms (*Dugesia* sp.) in the test tanks, and suspected a relation between their presence and amphipod survival during the first 30 days. We therefore performed static tests (two with each amphipod species) in four fingerbowls, each with test water containing four flatworms and five newborn gammarids; as controls, we placed five gammarids in each of four additional fingerbowls with test water but without flatworms. All of the newborn gammarids in the bowls with flatworms but none in the bowls without flatworms died within 24 h. Tested under the same conditions, juvenile gammarids (less than 30 days old) had a 70% mortality after a 96-h exposure to the flatworms. Thus, the presence of flatworms probably contributed to the greater early mortality in test 1. Jenio (1972) reported that flatworms preyed on the three species of gammarids he tested (*G. minus pinicollis, G. pseudolimnaeus,* and *G. troglophilus*). If these predators could be excluded from the test tanks, the early mortality of young amphipods would probably be decreased.

In test 2, mean survival from first copulation to death was greater for *G. lacustris* (108 days) than for *G. pseudolimnaeus* (63 days), indicating that these test conditions were more favorable for *G. lacustris*. The lower survival time for *G. pseudolimnaeus* does not indicate a shorter life span than that of *G. lacustris* however, since 67% of the total mortality for both species occurred within 60 days and mortality of both species was low and about equal for animals of about this age in test 1. Of the *G. pseudolimnaeus* in test 2, females died first in seven tanks and males died first in five. In 10 of the test tanks containing *G. lacustris* the female died first, and in one the male died first. In one tank both the male and female were still alive after 218 days (more than twice the mean duration of the other *G. lacustris* pairs); observations were then discontinued. The results differ from the findings of Hynes and Harper (1972), whose field studies with *G. lacustris* indicated that the males tended to die before the females.

In test 2, at 20 weeks there was no significant difference in fecundity between *G. lacustris* and *G. pseudolimnaeus*, so the difference in the number of young produced was presumably due to the greater survival of *G. lacustris*. However, at the end of test 1 (20 weeks), *G. lacustris* had more (but not significantly more) broods per female than did *G. pseudolimnaeus*. Also in test 1, both species produced more young per female and more broods per female than in test 2 after 20 weeks. Thus, when several pairs of amphipods were held together, conditions were apparently more favorable than when individual pairs were held alone. The greater number of broods per female in test 1 might have been a result of the greater availability of sexual partners. Strong (1973), working with *Hyalella azteca*, found that a female may be amplexed by more than one male. Such behavior could possibly increase chances of egg fertilization.

Of the two species, we prefer *G. lacustris* for testing

because its distribution is wider, making it easier to obtain (Holsinger 1972) and it is also larger. On the average, at the end of the experiment *G. lacustris* was considerably longer than *G. pseudolimnaeus*—the males by 2.4 mm (26%) and females by 1.0 mm (22%). The larger size of *G. lacustris* facilitated 30-day counts, observation and recovery of gravid females, and counts of young released.

References

American Public Health Association, American Water Works Association, and Water Pollution Federation. 1976. Standard methods for the examination of water and wastewater. 14th ed. Washington, D.C. 974 pp.

Arthur, J. W., and J. G. Eaton. 1971. Chloramine toxicity to the amphipod *Gammarus pseudolimnaeus* and the fathead minnow (*Pimephales promelas*). J. Fish. Res. Board Can. 28:1841-1845.

_____, A. E. Lemke, V. E. Mattson, and B. J. Halligan. 1973. Toxicity of sodium nitrilotriacetate (NTA) to the fathead minnow and an amphipod in soft water. Water Res. 8:187-193.

_____, and E. N. Leonard. 1970. Effects of copper on *Gammarus pseudolimnaeus, Physa integra,* and *Campeloma decisum* in soft water. J. Fish. Res. Board Can. 27:1277-1283.

Holsinger, J. R. 1972. The freshwater amphipod crustaceans (Gammaridae) of North America. Biota of freshwater ecosystems. Identification Manual No. 5, U.S. Environmental Protection Agency, Washington, D. C. Water Pollution Series 18050 ELDO 4/72.

Hynes, H. B. N., and F. Harper. 1972. The life histories of *Gammarus lacustris* and *G. pseudolimnaeus* in southern Ontario. Crustaceana 3:329-341.

Jenio, F., Jr. 1972. The *Gammarus* of Elm Spring Union County, Illinois (Amphipods Gammaridae). Ph.D. thesis, Southern Illinois University, Carbondale. 182 pp.

Macek, K. J., K. S. Buxton, S. Sauter, S. Gnilka, and J. W. Dean. 1976a. Chronic toxicity of atrazine to selected aquatic invertebrates and fishes. U.S. Environmental Protection Agency, Ecol. Res. Ser. EPA-600/3-76-047. 49 pp.

_____, _____, S. K. Derr, J. W. Dean, and S. Sauter. 1976b. Chronic toxicity of lindane to selected invertebrates and fishes. U.S. Environmental Protection Agency, Ecol. Res. Ser. EPA-600/3-76-046. 49 pp.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicity studies. Water Res. 1:21-29.

National Academy of Sciences, National Academy of Engineering. 1973. Water quality criteria 1972. U.S. Government Printing Office, Stock No. 5501-05520. Washington, D.C. 594 pp.

Sanders, H. O. 1969. Toxicity of pesticides to the crustacean *Gammarus lacustris*. U.S. Fish Wildl. Serv., Tech. Pap. 25. 18 pp.

_____. 1972. Toxicity of some insecticides to four species of malacostracan crustaceans. U.S. Fish Wildl. Serv., Tech. Pap. 66. 19 pp.

Smith, W. E. 1973. Thermal tolerance of two species of *Gammarus*. Trans. Am. Fish. Soc. 102:431-433.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, New York. 481 pp.

Strong, D. R., Jr. 1973. Amphipod amplexus, the significance of ecotypic variation. Ecology 54:1383-1388.

Accepted 25 January 1978

END

DATE

FILMED

10-19-78

NTIS